Does Elevated CO_2 and Lighting Intensity Affect Antioxidation Capacity of Green Onions?

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INTRODUCTION

Living organisms constantly combat reactive oxygen species (ROS)formed via either endogenous or exogenous mechanisms. ROS has been implicated in more than 100 diseases[1] (e.g. cancer, cardiovascular disease, neurodegeneration, diabetes and etc.). Space travelers will inevitably encounter increased dosages of ionizing radiation that would produce excess ROS. Although all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages, it can be inefficient in the presence of excess ROS. Therefore, dietary intake of antioxidant compounds becomes important. In an effort to ensure space traveler's survival and well-being during long-term missions, it may be important to have a self-sustaining life support system consisting of quality crops that not only fulfill water and air revitalization functions, but also provide psychological benefits. In addition, such crops could act as a counter measure against the damaging effects of ionizing radiation. It is well known that some fruits and vegetables are rich in antioxidant phytochemicals. Although apple and grapefruit are high in antioxidants, system mass constraints make them impractical to grow. Green onion is currently under evaluation as a candidate salad crop for life support systems due to its growth characteristics, flavor and potential health promoting factors including antioxidation and antiproliferation activities. However limited power and space on ISS or Mars transit vehicles and potential planetary based habitats create a unique environment that may be low in light intensity and high in carbon dioxide (CO_2). These conditions may negatively impact the antioxidation capacity of the green onions. The objective of this study is to assess the effect of lighting intensity and atmospheric CO_2 level upon the antioxidant capacity of green onion (*Allium fistulosum* L. cv Kinka).

MATERIALS AND METHODS

Plant Cultivation

Green onions (*Allium fistulosum* L. cv Kinka) were hydroponically grown in ½ Hoagland's solution under cool white florescence lamps (CWF) in an environmental growth chamber with 50% relative humidity, a photoperiod of 16/8 light/dark, and 25 °C. The CO_2 and light treatments were as follows:

CO_2 Level (ppm)	Lighting Intensity (μmol/m²S or PPF)
400	150, 300, 450
1,200	150, 300, 450

Sampling and Extract Preparation

34 day old plants were sampled, immersed in liquid nitrogen after removal of the roots and freeze-dried. Freeze-dried tissue was ground to homogeneity; 30 mg of tissue was then extracted either with water or 75% ethanol three times. Extracts (5 ml) were tested for total antioxidant activity (TAA) on the same day of extraction.

TAA Determination by ABTS Radical Cation Decolorization Assay[2]

ABTS was dissolved in water to a 7 mM concentration, and reacted with 2.45 mM potassium persulfate (final concentration) to form an ABTS radical cation. The reaction mixture was held at room temperature in the dark for 12-16 hr before use. The ABTS radical cation stock was diluted in 50% ethanol to an absorbance of 0.7-0.8 at 734nm. Two ml of this working solution was transferred into a cuvette and equilibrated at 30 °C using a Peltier Temperature Module for a spectrophotometer. Appropriate sample volume was added and mixed swiftly. Absorbance at 734 nm was taken before the addition and exactly 1 min after initial mixing. The percentage inhibition of absorbance was calculated and plotted as a function of sample volume or concentration. TAA was expressed as Trolox Equivalent Antioxidant Capacity (TEAC), that is the amount of plant material or liquid extract required to give the same percentage inhibition of absorbance of the radical cation as 1 mM Trolox at the end of 1 min. Therefore, the lower the TEAC value, the higher TAA of the sample.

Folin-Ciocalteu Method for Polyphenol Content Determination[3]

Polyphenol content of extracts was determined and converted to a tannic acid equivalent using a calibration curve.

RESULTS AND DISCUSSION

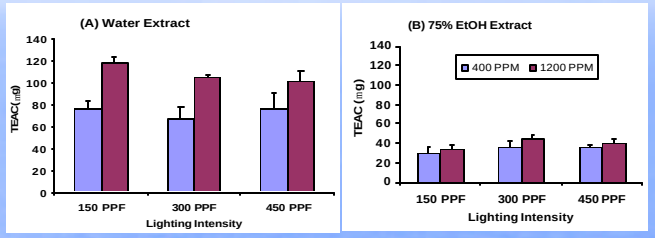

Fig. 1. TAA of Water Extract (A) and 75% Ethanol Extract of Green Onions Grown under Different Lighting Intensity and CO_2 Level

❖There was no significant difference in the antioxidation capacity of water or 75% ethanol extracts among onions grown under three light intensities (Fig. 1)

This may be explained by the greater carbon allocation to biomass than to antioxidants due to increased light intensity. As shown in Fig. 2. onion biomass linearly increased as lighting intensity increases when the CO_2 is not limited (1200 ppm CO_2). Consequently any potential enhancement of antioxidant phytochemicals may be diluted. It is also highly probable that the light intensities were too low to produce an observable difference; by comparison, sunlight has a maximum intensity of ~2000 PPF. Finally the effect of light intensity may be more pronounced in selective tissue types (such as the leaves) which actively accumulate antioxidants; such effects may be diluted when whole plant extracts are used as in this experiment. Analyses of the green leaves and pseudobulb separately indeed demonstrated that the TAA was higher in the green leaves than the bulb (TEAC was 85 ± 9 μg and 130 ± 10 μg for leaf and bulb respectively).

❖Elevated CO_2 resulted in significant decrease in TAA (higher TEAC) of green onions. This may be also due to the greater increase of biomass (at least 50% net increase in biomass at all lighting levels, Fig. 2), thus diluting any increase in antioxidants accumulated.

❖75% Ethanol Extract had higher antioxidant activity (lower TEAC) than that of the water extract.

This suggests that the phytochemicals which possess antioxidant activity in green onion are less polar, in other words, less soluble in water. Polyphenol is also known as a class compounds having radical scavenging capability. However, no difference was found in the total phenol content as tannic acid equivalent between two extracts (Table 1)

Table 1. Total Phenol Content (as Tannic Acid Equivalent mg/G DW) in Onion Extracts. Values are the average of four replicates and corresponding standard deviation

	400 ppm CO_2		1200 ppm CO_2	
Lighting Intensity	Water Extract	75% EtOH Extract	Water Extract	75% EtOH Extract
150 PPF	11.6±0.48	12.1±0.19	11.0±0.87	10.6±0.27
300 PPF	12.1±0.71	11.2±0.62	10.7±0.92	10.2±0.28
450 PPF	12.1±0.99	10.6±0.29	10.9±1.29	10.3±0.64

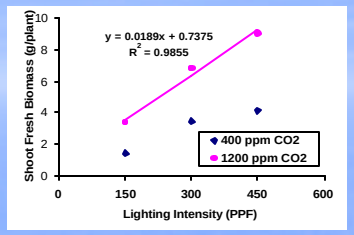

Fig. 2. Green Onion Shoot Biomass Increased as Lighting Intensity and CO_2 Levels Increased.

❖Where does green onion stand in terms of antioxidant activity?

Juice from various fruits and green onion purchased from a local grocery store was analyzed in the same manner, demonstrating that green onion juice has similar level of antioxidant activity as grapefruit and apple peel (Table 2).

Table 2. TEAC of Juices Expressed from Grocery Bought Fruits and Green Onion

Juice from Sources	TEAC (ul Juice)
Grapefruit	0.39
Apple Peel	0.40
Apple Flesh	3.36
Tangerine	1.19
Green Onion Stem	0.36
Green Onion Leaf	1.42

CONCLUSIONS

- There was no significant difference in the antioxidation capacity of water or 75% ethanol extracts among onions grown under three light intensities.
- Elevated CO_2 resulted in significant decrease in TAA (higher TEAC) of green onions.
- 75% Ethanol Extract had higher antioxidant activity (lower TEAC) than that of the water extract.
- Green onion juice has similar levels of antioxidant activity as grapefruit and apple peel

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